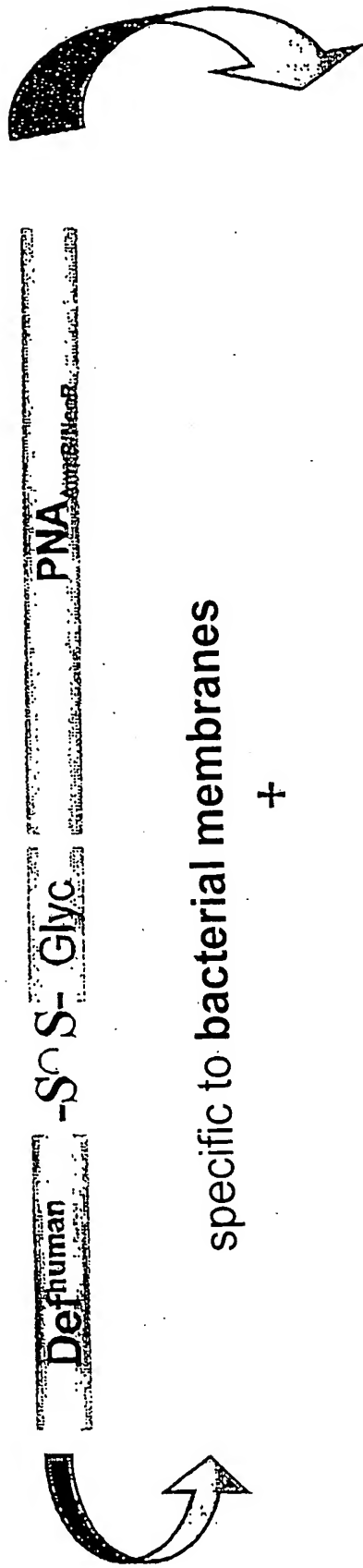


4/15

Strategies:



specific to bacterial membranes

anti-gene strategy directed specifically against bacterial genes

→ combined 'dual strategy'

Fig. 1

2/15

# Genome site of action - anti-gene PNA

DEFINITION Cloning vector pBR322, complete genome.  
 ACCESSION J01749 - Genbank  
 KEYWORDS ampicillin resistance; beta-lactamase; cloning vector; drug resistance protein; origin of replication; plasmid;  
 SOURCE Cloning vector pBR322.  
 ORGANISM Cloning vector pBR322  
 artificial sequence; vectors.

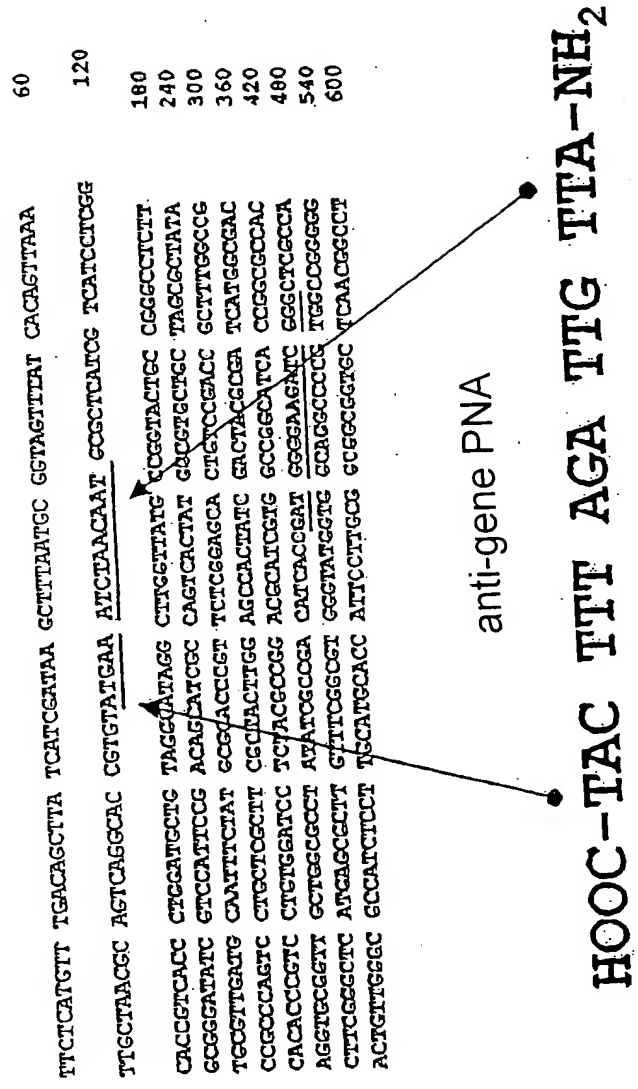


Fig. 2

3/15

Alignments Holin-Protein (Phagen) - *Transportprotein*

product="probable holin" (GMSE-1)  
[Endosymbiont bacteriophage may influence susceptibility to trypanosome infection in tsetse, Dale and Young]  
protein\_id="AAG50251.1"  
db\_xref="GI:12276078"  
translation="MPCLHLVVGWSSPGSALIREQAIGAGLAAWMTCLRGRYLGRGWKRKTTFDAAICALIAWF  
ARDGLALVGIDNQFSYLSSIIIVGYLGNDYLGALLRRRLEKKS GESNAPQ

product="holin protein" (Listeria innocua)  
protein\_id="CAA61518.1"  
translation="MMKMEFGKELLVYMTFLVVVTPVFVQAIKKTELIPSKWLPTVSILVGAILGALATSLDGSG  
SLATMIWAGALAGAGGTGLFEQFTNRAKKY GKDD

product="holin" (bacteriophage 80 alpha)  
specific\_host="Staphylococcus aureus RN450  
function="makes hole in membrane"  
protein\_id="AAB39698.1"  
db\_xref="GI:1763242"  
translation="MDINWKLRFKNKAVLTGLVGALFVFIKQVTDLFGLDLSTQLNQASAIIGAILTLLTGIGVIT  
DPTSKGVSDSSIAQTYQAPRDSKKEEQVQVTKWSSQDSSLTPELSAKAPKEYDTSQPFTDASNDVGFDVN  
EYHHGGGDNASKIN

product="holin" (Staphylococcus bacteriophage phi 11)  
note="ORF3; structural homologue of holin"  
protein\_id="AAA99522.1"  
db\_xref="GI:511841"  
translation="MDINWKLRFKNKAVLTGLVGALFVFIKQVTDLFGLDLSTQLNQASAIIGAILTLLTGIGVIT  
DPTSKGVSDSSIAQTYQAPRDSKKEEQVQVTKWSSQDSSLTPELSAKAPKEYDTSQPFTDASNDVGFDVN  
EYHHGGGDNASKIN

product="putative holin 1" (Streptococcus pneumoniae bacteriophage MM1)  
function="lysis protein"  
protein\_id="CAC48114.1"  
db\_xref="GI:15074937"  
translation="MKIEFFNFLRSVIQTEDGLVLYALALIVSMEIDFVTGTIAAIIINPDIEYKSKIGINGLLRKISGV  
LLLMILIPASVLLPEKTGFALYSICLG YIAFTFQSLIENYRKLKGNVTLFQPIVKVFQRLLEKDDDDTKKGE

gene="orf87a" (Streptococcus thermophilus bacteriophage Sfi21)  
product="holin"  
protein\_id="CAA64941.1"  
db\_xref="GI:2292749"  
translation="MKKRKKKMNFKLRLQNKATLVALISAVFLMLQQFGLHVPNNIQQINTLVGILVILGIITDP  
TTKGIADSERALSIIQPLDDKEVY

gene="hol500" (Bacteriophage A500);(Listeria monocytogenes)  
protein\_id="CAA59363.1"  
/db\_xref="GI:853745"  
/translation="MMKMEFGKELLVYMTFLVVVTPVFVQAIKKTELIPSKWLPTVSILVGAILGALATSLDGSG  
SLATMIWAGALAGAGGTGLFEQFTNRAKKY GKDDK

product="holin" (Bacteriophage PL-1)  
protein\_id="BAA96748.1"  
translation="MQNELLQVLAIAFVIAPIITGTFEIKRYTPAEGKLLPVLSIGTG  
ILLACVWAMAFGHLPLIGAYALAGMLSGLASVGVYQIVKPNEEVK

Fig. 3(1)

4/15

gene="lydA" (Bacteriophage P1) (enterobacteriae) ✓  
codon\_start=1  
product="holin"  
protein\_id="CAA61014.1"  
db\_xref="GI:974764"  
translation="MLDTQELAPVAIALLLSVIGGIGTFLMDVRDGRQSGNLLGLVTEIFVAVTAGAVAYLLGQH  
EGWELSITYLMVTIASNNGHEVISGMKRVNIDSILNVLTSV VKKGGGK

gene="S" (Bacteriophage H-19B)  
note="similar to Bacteriophage 21 lysis gene S, encoded by GenBank Accession Number M65239" /  
product="putative holin protein"  
protein\_id="AAD04658.1"  
db\_xref="GI:2668771"  
translation="MEKITTGVSYTTSVAVGTGYWLLQLLDKVSPSQWVAIGVLGSLFLGLLTYLTNLYFKIREDR  
RKA VRGE

gene="hol" (Bacteriophage A118) ✓  
function="forms unspecific lesions into cytoplasmic membrane prior to lysis"  
specific\_host="Listeria monocytogenes"  
note="ORF24; two products may be translated from this gene (hol-96 and hol-93)"  
product="holin"  
protein\_id="CAB53810.1"  
db\_xref="GI:5823622"  
translation="MIEMEFKGKELLVYMTFLVVVTPVFVQAIKKTELVP SKWLPTVSILIGAILGALATFLDGSGS  
LATMIWAGALAGAGGTGLFEQFTNRSKKYGEDDK

gene="Hol" (Lactobacillus casei bacteriophage A2)  
product="putative holine" ✓  
protein\_id="CAB87385.1"  
db\_xref="GI:7573220"  
translation="MKJNWKVAVLSVKFWLALVPAALLVVQTAAAVFGYNWDFANLGKELTAVINAVFALLTI  
VGVAVDPTTEGVSDSQALAYPALITTKAAKIKSLEDQIKALQADKAADQATSAASEVVPETSSAAPAE  
SAPESVAPVASEEVK

gene="Hol" (Lactobacillus bacteriophage phig1e) ✓  
product="holin"  
protein\_id="CAA66751.1"  
db\_xref="GI:1926366"  
translation="MDIITSLNLATAGELALISFFIGVIVQAIKKTKGVKNTYLPFISMGIGILAGLAAVVVTKDTN  
YLN GAVAGLIVGAATSGITDGLSVGTSAVTTAKATKDAAKTAAITQAVLNSINTTKSSDTTQVANTSN  
TEGGSTSETQK

product="holin" (Lactobacillus delbrueckii subsp. lactis bacteriophage LL:H) ✓  
protein\_id="AAC00556.1"  
db\_xref="GI:623083"  
translation="MTLIDWFNLIVAIGTIALAVVASVYVHLKAKIDTKTAAGKAFDLVGKLAVWAVNEAEHSQ  
DGGAAKREFAAKLISDQLKAKGITGIDEKMOVYGA VETAWKEA IENVK

product="holin protein" (Lactococcus phage c2) ✓  
protein\_id="AAD20611.1"  
db\_xref="GI:4426933"  
translation="MIETLRAIGLVVFMQLLSLALEFIDTGTLPKPSVRKRIAVELMVL

gene="hol" (bacteriophage phiAM2) ✓  
note="hydrophobic pore-forming protein"  
product="holin"  
protein\_id="AAG24367.1"  
db\_xref="GI:10880732"  
translation="MFFNNKFYNVIKWA VLTALPALS VFIGVIGKAYGWGGTDLAHTLNAFTVFLGTLAGVSAV  
KYNSQPNDTKENK

5/15

product="holin"  
protein\_id="AAG24367.1"  
translation="MFFNNKFYNVIKWA VLTALPALS VFIVIGKAYGWGGTDLAIITLNAFTVFLGTLAGVSAV  
KYNSQPNDTKENK

product="holin" (Bacteriophage Tuc2009)  
protein\_id="AAA32614.1"  
db\_xref="GI:496282"  
translation="MNQINWKLRLKSKAFWLALLPALFLLIQAIGAPFGYKWDFVILNQQLAAVVNAAFALLAI  
VGVVADPTTSGLGSDRVLNKDKSEENK

product="holin" (Bacteriophage TPW22)  
function="formation of non-specific lesions in the cytoplasmic membrane"  
protein\_id="AAF12704.1"  
db\_xref="GI:6465904"  
translation="MNQINWKLRLKSKAFWLALLPALFLLIQAIGASFGYKWNFVILNQQLAAVVNAAFALLAI  
VGVVADPTTSGLGSDRVLNKDKSEENK

product="holin" (homology to Orf78 of phage HP1 and gene S of phage P21)  
protein\_id="AAC45168.1"  
db\_xref="GI:915370"  
translation="MRFNMLKNSETTGAYVGSIAIATYSGFTLADWAAIFGILFGLFT M LINWYYKNK  
EIKLKETALKQKIDLKEGDHE

product="holin" (Bacillus phage GA-1)  
function="host cell lysis, holin formation"  
protein\_id="CAC21535.1"  
db\_xref="GI:12141291"  
translation="MFEFFHSLMETDDTKVYFLLGIIGVLNIVDFFFGFINAKFNKSLAYKSSKTIDGIMRKMFKFTI  
MAILFIPVSVLMPPIGLGALYVFYFGYIYAELNSILSH LKLSGDKETEVFLDFINTFFNSTKGDKKDD

gene="hol187" (Staphylococcus phage 187)  
function="forms pores to allow access of lysin to CW"  
product="holin protein Hol187"  
protein\_id="CAA69023.1"  
db\_xref="GI:2764984"  
translation="MLMVIMVGNVGIYLTIFLIDTGTLRHQATQEIWHGIDILKGLKC LETLLILSLNQVI

gene="s" /function="holin" (Shigella dysenteriae)  
product="S protein"  
protein\_id="CAC05628.1"  
db\_xref="GI:9955825"  
translation="MYQMEKITTGVSYTTSVAVGMGYWFLQFLDRVSPSQWAAIGVLGSLLFGLLTYLTNLYFKI  
REDRRKAARGE

gene="E"  
protein\_id="CAA42879.1"  
db\_xref="GI:14781"  
db\_xref="SWISS-PROT:P31280"  
translation="MERWTLDDILAFLLLLSLLPSLLIMFIPSMYKQHASLWKARSLAKTLSMASSARLTPLSSS  
RTPCVLKQDSKKL

gene="xhlB" (B. subtilis DNA (28 kb PBSX/skin element region))  
product="holin-like protein"  
protein\_id="CAA94048.1"  
db\_xref="GI:1225964"  
db\_xref="SWISS-PROT:Q99163"  
translation="MNTFDKGTVIRTVLLIALINQTMMLGKSPLDIQEEQVNQLADALYSAGSIAFTIGTTLAA  
WFKNNYVTEKGKKQRDLLRDNNLTK

Fig. 3(3)

6/15

gene="bhlA" (Bacillus subtilis 168 prophage) ✓  
product="holin-like protein"  
protein\_id="AAC38301.1"  
db\_xref="GI:2997596"  
translation="MEMDITQYLSTQGPFVLCWLLFYVMKTSKERESKLYNQIDSQNEVLGKFSEKYDVVIE  
KLDKIEQNFK

gene="bhlB" (Bacillus subtilis 168 prophage) ✓  
product="holin-like protein"  
protein\_id="AAC38302.1"  
db\_xref="GI:2997597"  
translation="MFENIDKGTIVRTLALLAALLNQIMVMLGKAAFIINEEDINHLYDCLYTIFTIVFTTSTTTAA  
WFKNNYITAKGKKQKQVLKKENLFK

gene="hol" (Bacteriophage phi-Ea1h) ✓  
specific\_host="Erwinia amylovora"  
function="pore formation"  
product="holin"  
protein\_id="CAC17008.1"  
db\_xref="GI:11342496"  
translation="MRKIYVVIITIVVAGLIWAFIATQVNTGVTSKRQEDALAVSEANVGIGKEAKDQGEQATK  
RADVAKEQORTHQINQLKDKLHEKAESYDSIPLSPSDVDILC RAYRSTDPVCSPVTKSD

Fig. 3(4)

7/15

### Alignments Lysis-Protein (Phagen)

product="lysis protein" (Phage phiX174)✓  
function="host cell lysis"  
protein\_id="CAA84691.1"  
translation="MVRWTLWDTLAFLLLSLLPSLLIMFIPSTFKRPVSSWKALNLRKTLLMASSVRLKPLNCS  
RLPCVYAQETLTFLLTQKKTCVKNYVQKE

Fig. 3(5)

I / E. Coli<sub>competent</sub> XL - 10 GOLD + PNA-Probe (500 nM)

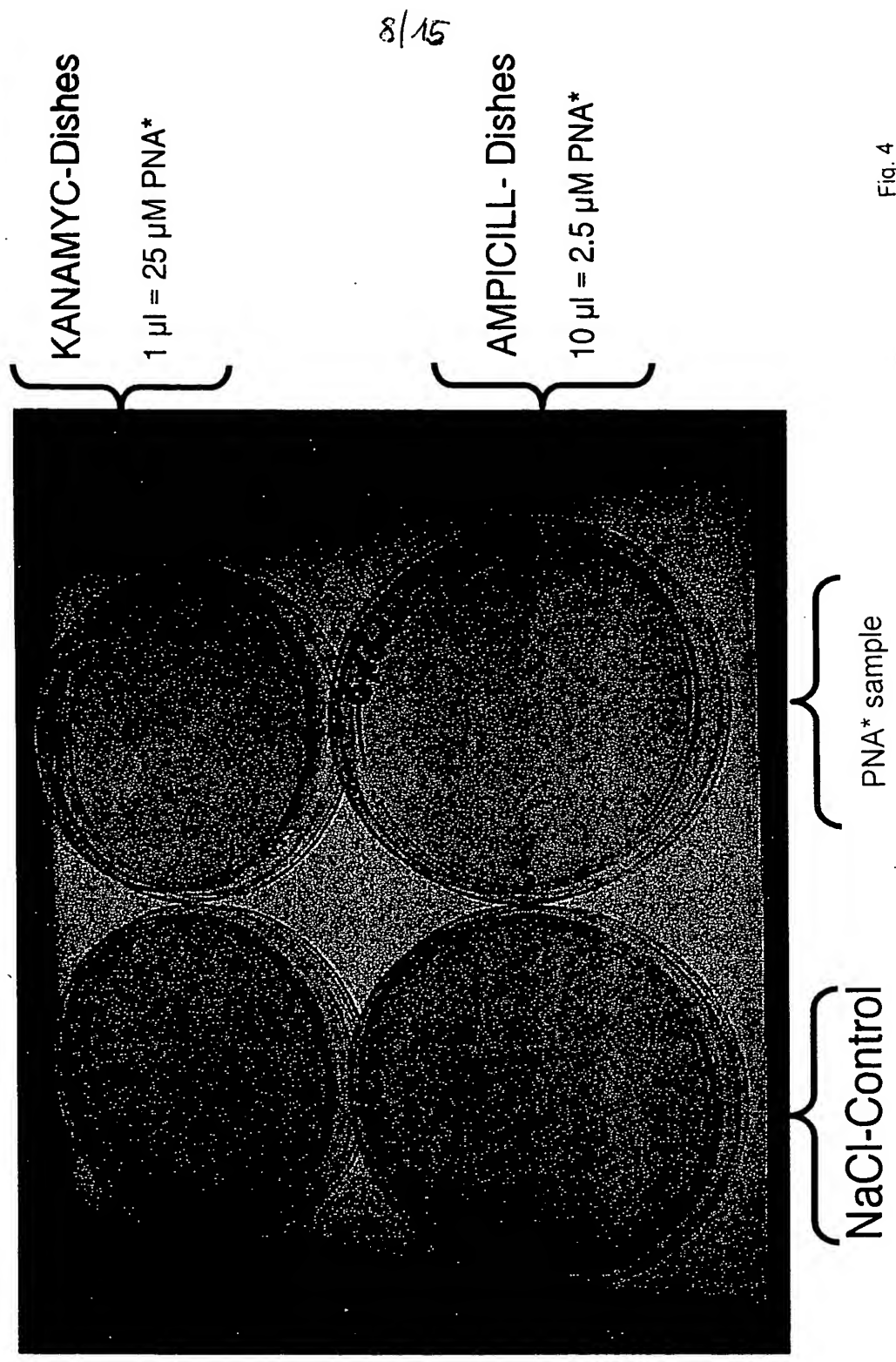


Fig. 4

\* peptide nucleic acid transport complex

intact bacteria + incubation with  $\alpha$ -kan-PNA

E.K.PNA-Konstr.  
 $\approx 2 \mu\text{M}$

9/15

$\approx 2 \mu\text{M}$

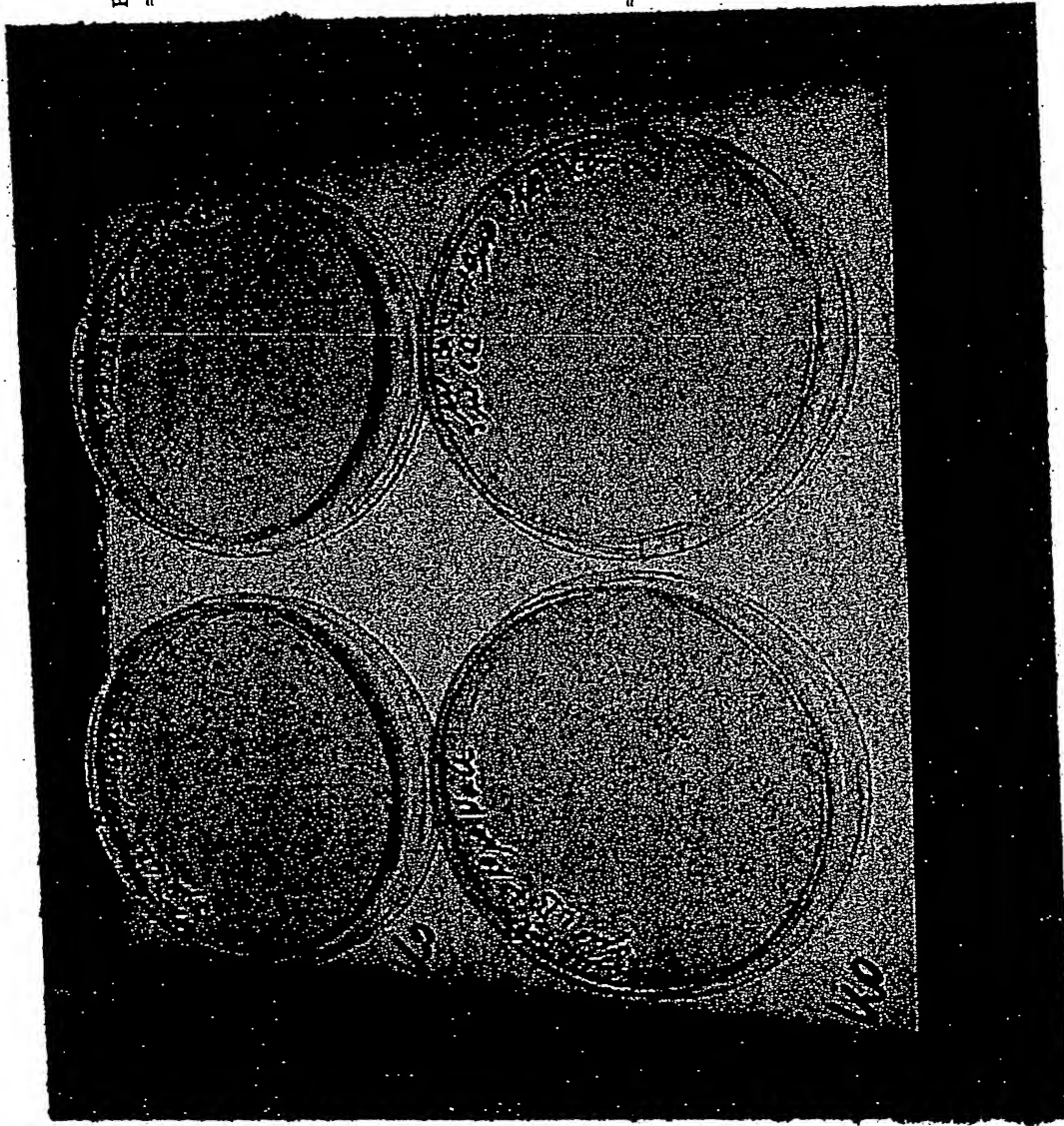
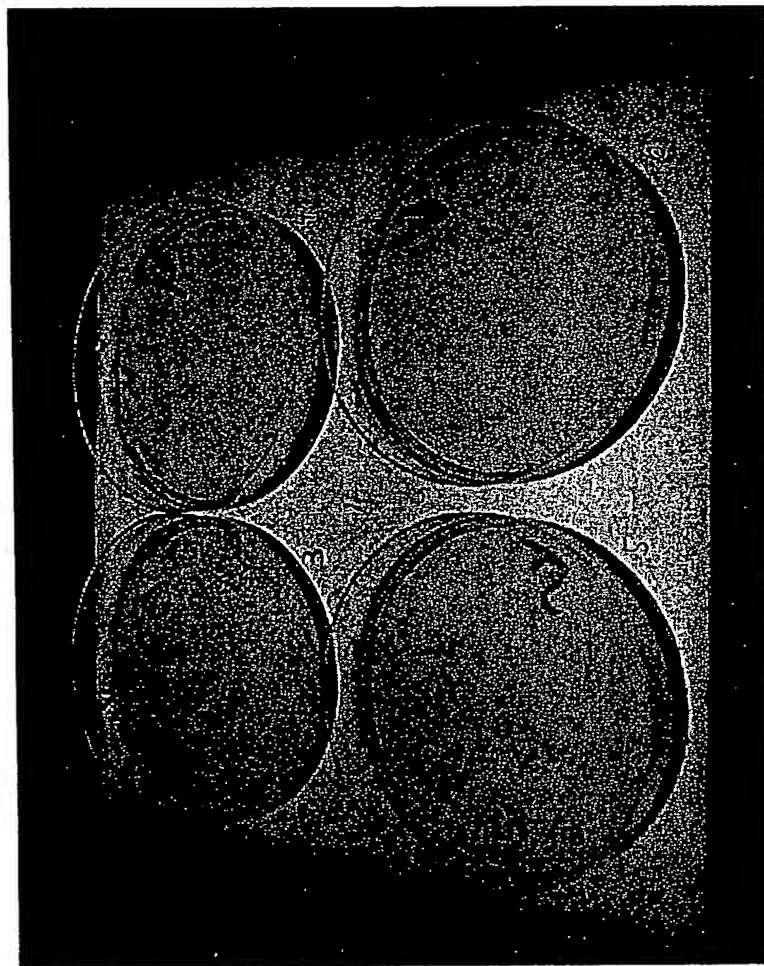


Fig. 5

II /

E. Coli<sub>intact</sub>/Kanresist

Optimizing Number of E. Coli per plate  
serial dilution of E. Coli by spectrophotometric measurements 600 nm



- 10/15
- 1) 1 : 10
  - 2) 1 : 100
  - 3) 1 : 1000
  - 4) 1 : 10000
  - 5) 1 : 100000
  - 6) 1 : 1000000

Serial Dilution of intact E. Coli to optimize the antibacterial incubation

Fig. 6

\* peptide nucleic acid transport complex

11/15

III K1-Control E. Coli<sub>intact/Kanresist</sub>



Kanamycine Dishes

- 3 PNA<sub>Ampresistgene</sub> (2,5  $\mu$ M) - 10  $\mu$ l      2 PNA<sub>Kanresistgene</sub> (25  $\mu$ M) - 1  $\mu$ l  
Identic: Bacter. Nmb; identic. PNA conc

Fig. 7

IV / analogue III - K1-Control E. Coli<sub>intact</sub>/Kanresist

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12/15

4 PNA<sub>Ampresistgene</sub> (2,5  $\mu$ M) - 10  $\mu$ l  
1  $\mu$ l E. Coli + 10 10  $\mu$ l PNA  
in LB medium

Fig. 8

\* peptide nucleic acid transport complex

13/15

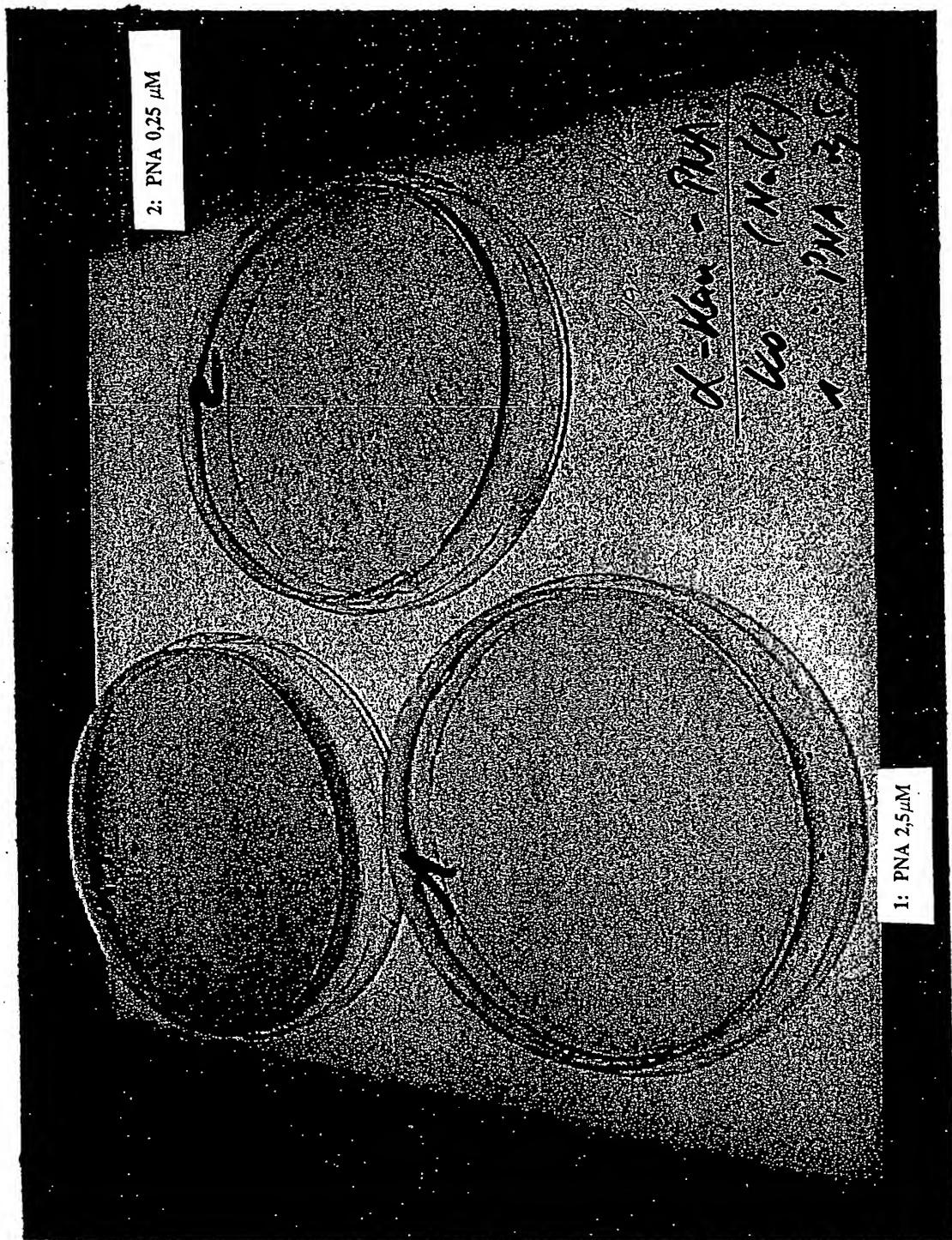


Fig. 9

14/15

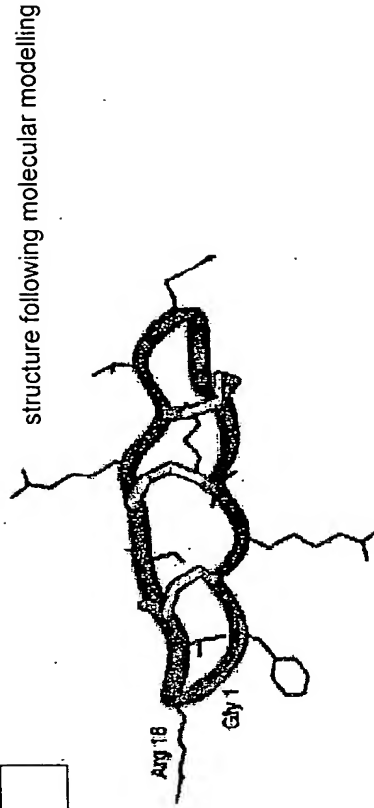
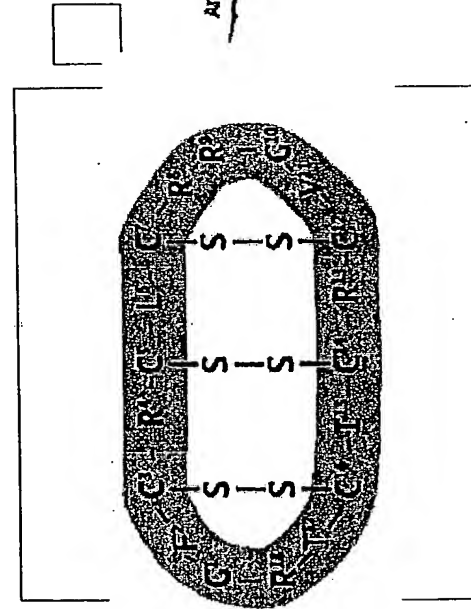
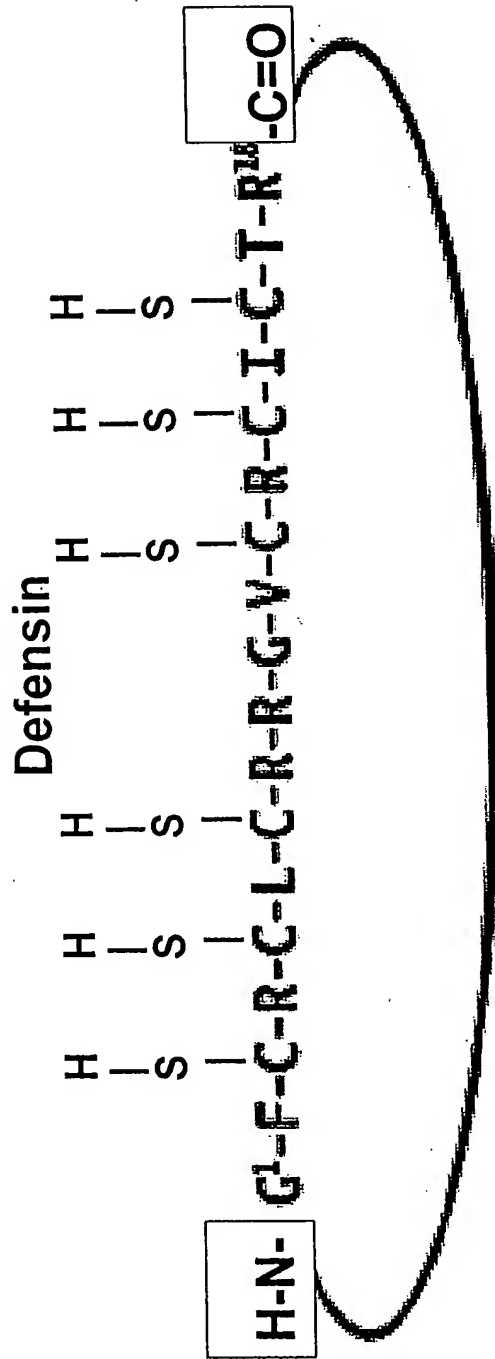
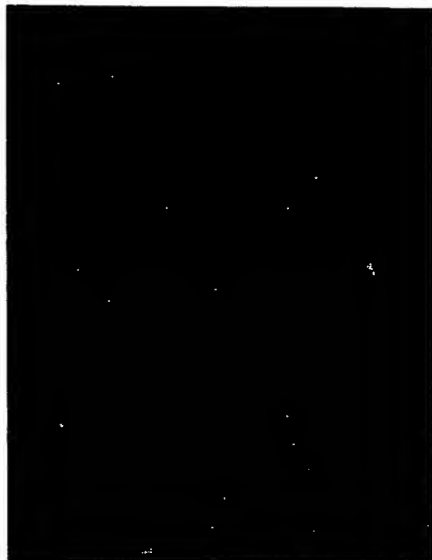


Fig. 10

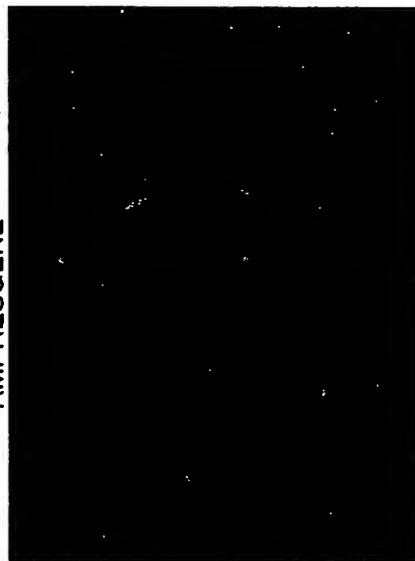
# Test for NO<sub>1</sub>-Toxicity in eucaryotic cells (HeLa)

HeLa  
Control  
(untreated)



A

PNA\* AMPRESENE - 25  $\mu$ M



PNA\* KANRESENE - 25  $\mu$ M



B

Fig. 11

\* peptide nucleic acid transport complex